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# BRAIN COMMUNICATIONS

## Amygdala hyperactivation relates to eating behaviour: a potential indicator of food addiction in Prader–Willi syndrome

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Prader–Willi syndrome is a rare neurodevelopmental genetic disorder characterized by various endocrine, cognitive and behavioural problems. The symptoms include an obsession for food and reduced satiety, which leads to hyperphagia and morbid obesity. Neuropsychological studies have reported that Prader–Willi patients display altered social interactions with a specific weakness in interpreting social information and responding to them, a symptom close to that observed in autism spectrum disorders. In the present case-control study, we hypothesized that brain regions associated with compulsive eating behaviour would be abnormally activated by food-related odours in Prader–Willi syndrome, as these can stimulate the appetite and induce hunger-related behaviour. We conducted a brain imaging study using the olfactory modality because odours have a high-hedonic valence and can cause stronger emotional reactions than other modalities. Further, the olfactory system is also intimately associated with the endocrine regulation of energy balance and is the most appropriate modality for studies of Prader–Willi syndrome. A total of 16 Prader–Willi participants were recruited for this study, which is a significant achievement given the low incidence rate of this rare disease. The second group of 11 control age-matched subjects also participated in the brain imaging study. In the MRI scanner, using an MRI-compatible olfactometer during 56 block sessions, we randomly presented two odours (tulip and caramel), which have different hedonic valence and a different capacity to arouse hunger-related behaviour. Our results demonstrate that Prader–Willi participants have abnormal activity in the brain reward system that regulates eating behaviour. Indeed, we found that these patients had right amygdala activity up to five times higher in response to a food odour (caramel) compared with the tulip odour. In contrast, age-matched control participants had similar activity levels in response to both odours. The amygdala activity levels were found to be associated with the severity of the hyperphagia in Prader–Willi patients. Our results provide evidence for functional alteration of the right amygdala in Prader–Willi syndrome, which is part of the brain network involved in food addiction modulated by the ghrelin and oxytocin systems, which may drive the hyperphagia. Our study provides important new insights into the functioning of emotion-related brain circuits and pathology, and it is one of the few to explore the dysfunction of the neural circuits involved in emotion and addiction in Prader–Willi syndrome. It suggests new directions for the exploration and remediation of addictive behaviours.

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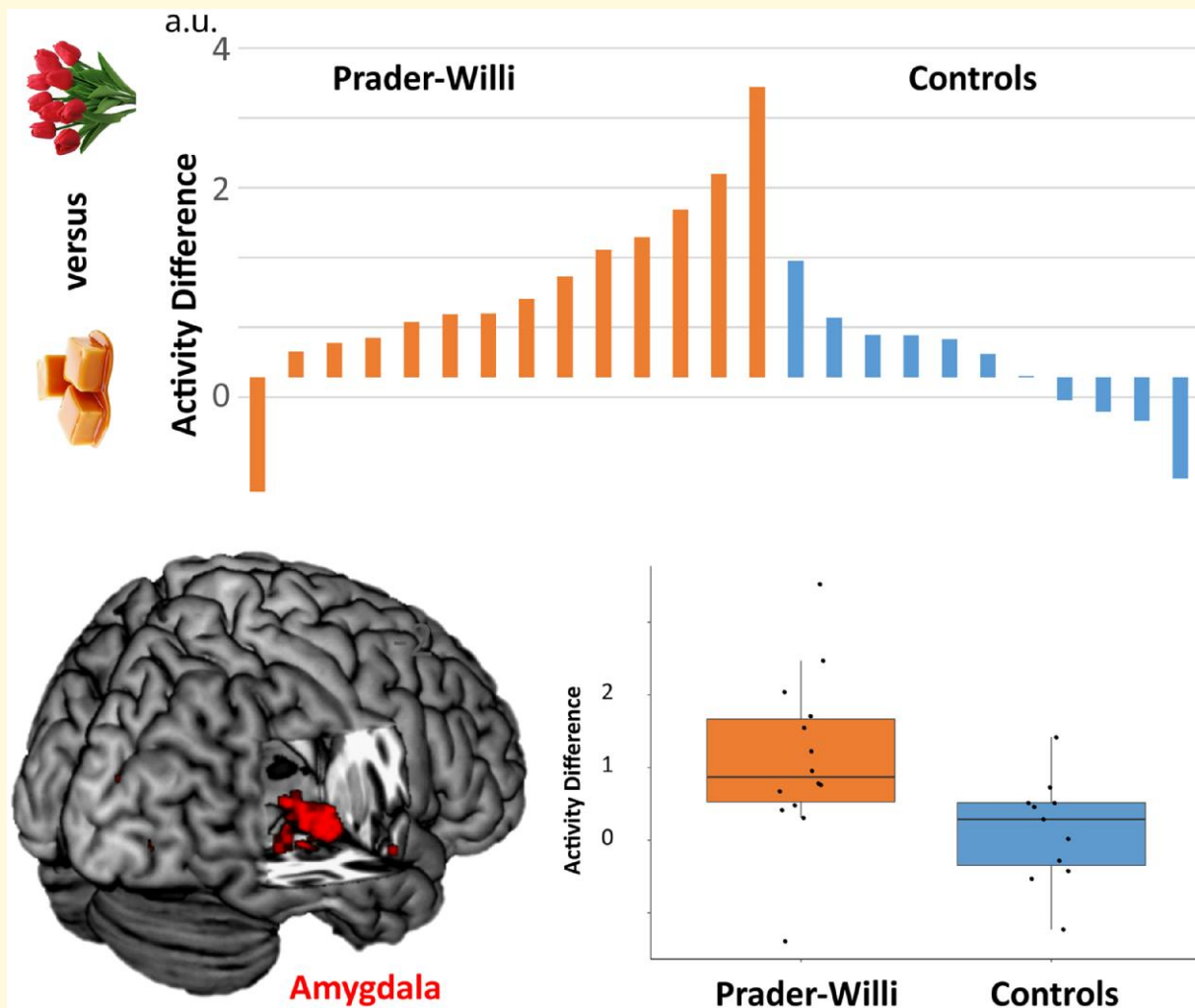
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**Keywords:** Prader–Willi; hyperphagia; compulsive; amygdala; food addiction

**Abbreviations:** ASD = autistic spectrum disorders; BMI = body mass index; DEL = deletion; fMRI = functional MRI; HQ = hyperphagia questionnaire; HQ-CT = hyperphagia questionnaire, caregiver reported; OXT = oxytocin; PWS = Prader–Willi syndrome; ROI = region of interest; RT = reaction time; SD = standard deviation; TD = typically developed; UPD = maternal uniparental disomy

## Graphical Abstract



## Introduction

Prader–Willi syndrome (PWS) is a rare genetic neurodevelopmental disorder that results from the loss of gene expression of paternally inherited maternally imprinted genes of chromosome 15 at position q11–q13. In more than 55% of cases, there is a microdeletion (DEL) of this chromosome region that is inherited from the father; in 40–45% of cases, there is maternal uniparental disomy (UPD); and in <5% of

cases, there is an imprinting defect or translocation involving this region. In other words, PWS occurs when there is a loss of expression of paternally inherited alleles, which can be due to paternal chromosomal deletion, absence of paternal chromosome 15 (maternal disomy) or absence of normal paternal imprinting marks (imprinting defect).

Our team recently confirmed that the incidence of PWS is around one in 20 000 births,<sup>1</sup> similarly in France and other countries. PWS is characterized by hormone deficiencies

and cognitive impairments, with intellectual disabilities that are usually mild. The syndrome is associated with a range of psychiatric symptoms, including emotional dysregulation and social-cognitive deficits. There can also be other comorbidities, such as sleep-related breathing disorders, scoliosis, gastrointestinal disorders, dysautonomia, including hypersomnia, narcolepsy and catatonia.

PWS is characterized by a specific trajectory that involves nutritional, endocrine/metabolic and emotional/behavioural dimensions. There is a paradoxical sequence that starts with poor feeding with failure to thrive and anorexia, followed by unexplained, excessive weight gain and then hyperphagia which leads to severe obesity.<sup>2,3</sup> In adulthood, there is compulsive food searching, storing and hoarding. The constant drive for food is a major source of stress and anxiety for patients, families and caregivers, and can prevent socialization.<sup>4</sup> The hyperphagia could therefore be described as an addiction to food.<sup>5</sup> A PWS-specific hyperphagia questionnaire (HQ) has been developed,<sup>6,7</sup> which assesses three relevant subscores/dimensions: (i) behaviour, relating to the times, frequency, actions and bargaining to obtain food; (ii) drive, relating to the strong desire (impulse) to speak about and consume food, and difficulty in diverting attention and avoiding frustration/anger; and (iii) severity, relating to the extent to which food-related thoughts, words and actions invade daily life.

The PWS phenotype has been shown to relate to impaired hypothalamic development and function.<sup>3</sup> The hyperphagia may relate to abnormalities in oxytocin (OXT) and ghrelin, which are both linked to the hypothalamus. These hormones interact very closely and are involved in brain development and in regulating the dopaminergic reward system. OXT is secreted by hypothalamic neurons in the paraventricular and supraoptic nuclei, while ghrelin exerts most of its metabolic and appetite-regulating effects in the hypothalamus. In people with PWS as a group, the total plasma ghrelin levels are elevated from birth and throughout life.<sup>8</sup> There are two forms of ghrelin: acylated (fatty acid bound to the peptide chain) and un-acylated. Acylated ghrelin has a strong orexigenic effect and is therefore called the 'hunger hormone', whereas un-acylated ghrelin inhibits the effects of acylated ghrelin and has a resulting anorexigenic effect.<sup>9</sup> It is possible that the hyperghrelinemia in PWS may play a role in the obsessive food-related thoughts and addiction to food, as well as in increasing patients' appetites. The OXT system is also dysfunctional in patients with PWS. This hormone is known to be involved in emotion processing, and OXT administration has been found to increase trust in others and decrease sadness tendencies in patient with PWS.<sup>10,11</sup> It is possible that this hormone also plays a role in the hyperphagia in PWS.

Addictive behaviours involve different processes, such as incentive salience, stimulus-response habits, attentional bias, craving, executive functioning, and place- and emotional-conditioning.<sup>12</sup> The sensory systems play an important role in these, especially in the case of incentive salience. Indeed, it has been found that the activation of

sensory brain regions in response to drug-associated cues can predict relapse<sup>13</sup> and also correlates with craving, the severity of dependence and automatized motor responses to the cues.<sup>14</sup> The olfactory system plays an important role in nutrition and social behaviour, and there is evidence that it is linked to the endocrine regulation of energy balance.<sup>15</sup> Several studies have demonstrated that odours trigger an appetite for the relevant food,<sup>16-19</sup> a phenomenon known as sensory-specific appetite. In this way, odours can alert us to food in our environment and orient our appetite accordingly.<sup>20,21</sup> There is evidence that the olfactory system may play a role in eating disorders. For instance, it has been found that overweight children fail to regulate food intake after exposure to an intense smell of tasty food,<sup>22</sup> and patients with bulimia nervosa report a greater urge to binge eat after exposure to smell and taste cues.<sup>23</sup>

In this study, we investigated the effect of food-related or unrelated odours on the activity of brain regions involved in reward (see Reference<sup>24</sup> for review) in patients with PWS. We hypothesized that brain regions associated with compulsive eating behaviour, namely the basal ganglia, the extended amygdala and the prefrontal cortex, would be differentially activated in participants with PWS and controls. Despite the paucity of brain imaging studies in PWS, there are indications of dysfunction of these structures. Indeed, studies in patients with PWS show structural alterations<sup>25,26</sup> of cortical and subcortical regions (the ventral orbitofrontal, the anterior cingulate cortex and the caudate nucleus) implicated in emotional processing and regulation of behaviour. Similarly, there is evidence of altered functional connectivity at rest<sup>27</sup> of the prefrontal cortex and the basal ganglia or amygdala, which are associated with the severity of compulsive behaviour in patients with PWS.<sup>28</sup> We made the assumption that the high hedonic valence of the odours would optimally reveal any dysfunction in the addiction-related brain circuits, including the amygdala.

## Materials and methods

This clinical study (NCT0280437-33) was designed for adults with PWS and included brain imaging, neuropsychological assessments, the HQ and an HQ that was modified to be used for clinical trials [hyperphagia questionnaire, caregiver reported (HQ-CT)].

## Participants

A total of 16 participants with PWS were recruited for this study based on patient's availability. One of these was excluded because of poor performance on the olfactory test (50%, below 80% as indicated in the test documentation) and another was excluded because of technical problems in the MRI scanner. The analyses were therefore carried out for 14 patients, 8 of whom had DEL and 6 of whom had UPD. The average age was  $25 \pm 9$  (SD) years, and there were six women. The body mass index (BMI) of the PWS

**Table 1** Characteristics of the participants with PWS

| Patients | Genetic subtype | Sex | Age (years) | Total score HQ | Total HQ-CT | BMI  |
|----------|-----------------|-----|-------------|----------------|-------------|------|
| 1        | UPD             | F   | 19          | 11             | 0           | 23.0 |
| 2        | DEL             | F   | 19          | 17             | 5           | 25.6 |
| 3        | UPD             | F   | 26          | 27             | 18          | 32.6 |
| 4        | DEL             | M   | 19          | 27             | 21          | 21.6 |
| 5        | DEL             | F   | 19          | 21             | 14          | 29.1 |
| 6        | DEL             | F   | 38          | 26             | 18          | 42.3 |
| 7        | DEL             | M   | 20          | 39             | 32          | 38.6 |
| 8        | UPD             | M   | 29          | 28             | 21          | 30.3 |
| 9        | DEL             | F   | 19          | 40             | 23          | 26.5 |
| 10       | UPD             | M   | 19          | 38             | 21          | 25.4 |
| 11       | UPD             | M   | 23          | 37             | 21          | 31.8 |
| 12       | DEL             | M   | 38          | 19             | 4           | 43.6 |
| 13       | DEL             | M   | 18          | 19             | 4           | 24.1 |
| 14       | UPD             | F   | 46          | 38             | 20          | 41.2 |

participants was  $31.1 \pm 7.5$  (SD). Eleven controls were recruited for the study; the average age was  $26 \pm 3$  (SD) years, and there were six women (see [Supplementary Table 1](#)). The differences between PWS participants were not significant for age (Mann–Whitney,  $W = 122.5$ ,  $P = 0.22$ ) and sex ratio ( $z$ -proportion test,  $z = 0.68$ ,  $P = 0.50$ ). The characteristics of the patients are shown in [Table 1](#).

All of the patients participated in the study during routine follow-up visits to our centre in Toulouse. Height, weight, BMI, evaluation of eating behaviour using the HQ, olfactory testing and functional MRI (fMRI) were all carried out in a 1-day visit. Genetic subtypes were retrieved from the patients' medical files. Sex- and age-matched controls were recruited for the study via a public announcement.

We deliberately chose not to recruit overweight people as controls for this study. Our group found that ghrelin and hyperphagia are more important hallmarks of PWS than obesity.<sup>29</sup> Indeed, even without increased BMI, patients with PWS display hyperphagia. PWS constitutes a broad spectrum of symptoms and shares some common points with other pathologies, such as autistic spectrum disorders (ASD). ASD patients, like PWS, present substantial deficits in processing social communication information and show signs of compulsive behaviour. This type of patient could very well have been compared to the processing alterations observed in the PWS, especially concerning the olfactory modality and the emotional processing of odours.<sup>30</sup> In the present study, we first wanted to determine the presence of an alteration in the brain network for processing odours with emotional valence. A comparison with other pathologies, such as the compulsive behaviour of obese people, will constitute a second step in order to define if these alterations are specific to PWS.

## Olfactory test

A standardized clinical test<sup>31</sup> was administered to evaluate the ability to recognize different odours. This involved presenting participants with sticks with different odours and

asking them to complete a four-alternative forced-choice task (a card with four names of odours). Twelve different odour sticks were used; each was presented once. The odours were the following: orange, leather, cinnamon, peppermint, banana, lemon, liquorice, coffee, cloves, pineapple, rose and fish. They did not include the odours presented in the fMRI examination to avoid their recollection.

## Visual detection task

A visual detection task was administered, as food-related behaviour may be influenced by visual information as well as olfactory stimulation. For this task, pictures were used from the International Affective Picture System.<sup>32</sup> The first set of pictures was of human faces; the second set included everyday objects and food; the third set contained the same images as the second set, but a Fourier transformation was applied to randomize the spectral content. There were a total of 12 images in each set, and these were presented in a random order. Participants were asked to respond as quickly as possible when they saw a picture by pressing a response key. Images were presented until the participants had responded. The stimuli were presented and responses were recorded using E-Prime software.

## fMRI procedure

The participants wore a mask and were asked to focus on odours that were sent through the mask. Two different olfactory stimuli were used: an odour related to food and an odour unrelated to food. For the former, we chose a caramel odour, because individuals with PWS have a preference for sweet foods; for the latter, we chose a tulip odour, because these flowers are not associated with food. The two odours were presented randomly, and there was no auditory or visual cue to alert participants to the onset of an odour. Each odour was presented in blocks that lasted 24 s. Within a block, the odour was presented for 4 s, followed by clean ambient air for 2 s (a purge). This was repeated four times. The clean air was used to prevent olfactory bulb habituation to the odours. Between the blocks, clean air was sent for 12 s, and this was used as the baseline in the analysis.

In total, there were 56 blocks with the caramel odour, 48 blocks with the tulip odour and 52 clean air baseline recordings. The total acquisition time was 16 min, which was divided into two 8-min blocks with a small break in between. Since PWS patients have variable cognitive abilities, we did not want to add difficulty by asking for an active task during the imaging session. Thus, no task was imposed, subjects were free to move their eyes and were instructed to inhale the odours well. No information about the odours presented in the MRI scanner was given.

The odours were presented using an olfactometer that was constructed based on a design from the De Volder team.<sup>33</sup> This system has already been tested for use in MRI experiments.<sup>34</sup> The system involves delivering odours through a 5-m Teflon tube, which is directly connected to two vials

that contain the odours. The olfactometer was connected to a computer, which controlled the opening and closing of valves. A scuba tank was used to continuously supply air and send the odours into the mask. The synchronization of the olfactory stimuli with the fMRI data was carried out using software developed by the De Volder team.<sup>33</sup> According to the manufacturer,<sup>33</sup> the rise time in the mask for the odours at 5 Ba was 1 s; the Universal Serial Bus port was used to command the olfactometer under Windows 10.

## Image acquisition parameters

Blood oxygen level-dependent and anatomical images were acquired using a 3 T Philips ACHIEVA X-series MRI scanner. Anatomical images were obtained with T<sub>1</sub>-weighted gradient echo acquisition, a repetition time of 8.1 ms, an echo time of 3.7 ms, a flip angle of 8°, a field of view of 240 mm × 240 mm and a 1 mm × 1 mm × 1 mm resolution. For the blood oxygen level-dependent signal images, T<sub>2</sub>\* echo planar imaging was used with a repetition time of 3 s, an echo time of 30 ms, an acquisition time of 3 s, a flip angle of 90°, a field of view of 240 mm × 240 mm, a voxel size of 3 mm × 3 mm × 3 mm and a matrix size of 80 × 80 pixels. Forty-five slices were acquired per volume with a total of 315 volumes; the first three volumes were discarded. The MRI session lasted about 30 min, 16 min of which were for the fMRI.

## fMRI acquisition and analysis

The MRI images were analysed using SPM8 (<https://www.fil.ion.ucl.ac.uk/spm/>). The blood oxygen level-dependent images were slice-time corrected, realigned to the first volume of each acquisition across fMRI volumes for each subject, normalized to the MNI152 template, and smoothed using an isotropic Gaussian filter with a full width at half

maximum of 6 mm. The high pass filter of 128 s was used; the default statistical parametric mapping (SPM) convolution with the canonical hemodynamic response function was applied. As the subjects were instructed to inhale each odour well, head movements were potentially related to the task precluding their usage as an independent covariate in the design matrix. Mean difference in movements between caramels and tulips in controls was 0.11 mm for translations and 0.14° for rotations, in patients, they were 0.33 mm and 0.29° correspondingly. Due to the high-quality fixation of the head in the camera, these movements were negligible compared to the size of the voxel (3 mm × 3 mm × 3 mm).

## Statistical analysis

We used the random-effect analysis, which was preceded by an estimation of the contrast maps (for caramel and tulip odours versus baseline) at the individual level with fixed-effect analysis. The contrast maps were further estimated in the paired *t*-test for the groups of PWS participants and controls. The ‘caramel–tulip’ contrast was compared between the groups with family-wise error (FWE) correction. No additional covariate of interest or no interest was included in the SPM analyses.

We specified two regions of interests (ROIs): the left and right amygdala, which are cytoarchitectonically defined. These ROIs were chosen because amygdala activity has been associated with the Yale Food Addiction Scale,<sup>35,36</sup> and also because amygdala activation has been observed for the anticipation of food.<sup>35</sup> The ROIs were defined using the SPM Anatomy Toolbox. The SPM small-volume correction was applied for the ROIs.

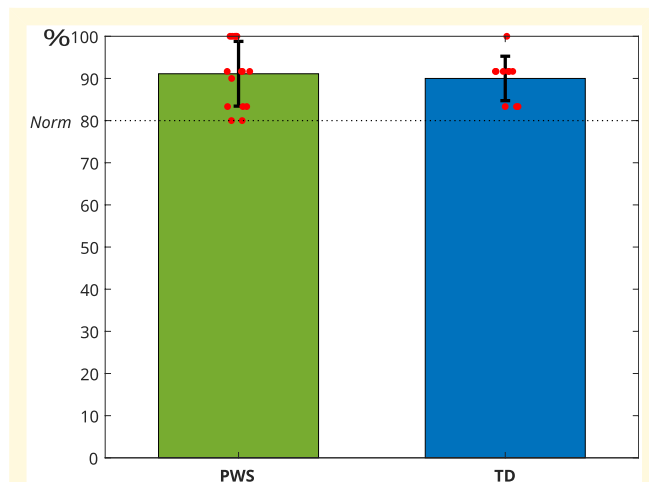
We used k-means clustering (R stats library), an unsupervised machine learning algorithm, to partition the subjects into groups according to their hyperphagia scores. This was carried out because of the heterogeneous patient profiles and the large number of clinical scores. The k-means algorithm identifies a fixed number of clusters within a data set, which aggregate together because of certain similarities in the scores. We predefined four clusters in order to approximate quartiles, which are widely used in the clinical literature. To stabilize the clustering results, 40 sets with random seeds were chosen for machine learning. The resulting clusters (groups) of subjects were used to assess group effects for brain activity with ANOVA.

## Results

### Behavioural results

For the olfactory test, the average scores were  $91 \pm 7.7\%$  for the patients with PWS and  $90 \pm 5.3\%$  for the typically developed (TD) controls (see Fig. 1). All participants obtained scores above the normal threshold value of 80%. The scores did not differ significantly between the two groups (*t*-test,  $P > 0.3$ ).

The visual test was run on the participants with PWS. The mean reaction time (RT) was  $837 \pm 732$  ms for the human



**Figure 1** Olfactory test results for the PWS and TD control groups. All of the participants in the fMRI study achieved a score above the cut-off of 80%. There was no significant difference between the participants with PWS and the controls.

face stimuli,  $726 \pm 594$  (SD) ms for the abstract images (with Fourier transformation),  $895 \pm 757$  ms for the everyday objects and  $731 \pm 737$  ms for the food images. PWS patients have typical RTs values that are extremely long (around 800 ms) compared to the relatively short RTs of control subjects due to the slower decision-making and a general slowdown in perception and motor reactions (see Reference <sup>37</sup>). The differences in RTs values according to the visual categories were not statistically significant (bootstrap,  $P > 0.05$ ). Similarly, no differences were found in the control group (see Supplementary Fig. 1). These results are in agreement with our previous study, where we found that there were no significant RT differences for different visual stimulus categories, including food, in a larger group of patients with PWS.<sup>38</sup> In that study, it was also found that the subjects with PWS were significantly slower than TD controls, as is usual for any task.

## Group analysis of the neuroimaging data

To analyse brain responses to food-related olfactory stimulation, we began by assessing the effect of the caramel odour in an exploratory whole-brain analysis. For the participants with PWS, this approach revealed that the most significant brain region activated more by the caramel odour than by the tulip odour had a peak in the region, which corresponded to the right amygdala [ $p_{\text{corr(FWE)}} < 0.05$ , size = 75,  $t = 5.99$ ,  $(x, y, z) = (21, -1, -13)$ ; Fig. 2A]. To confirm the location of this activation, the region was compared with the anatomical position of the amygdala according to the cytoarchitectonic atlas,<sup>39</sup> as illustrated in Fig. 2B. Figure 2C shows the brain activation together with the anatomical location of the amygdala, revealing an overlap of around 40%.

Though the peak was in the right amygdala, the cluster in the whole-brain analysis was too large, making it difficult to interpret. We, therefore, focused on the analyses using a ROI centred on the amygdala, as described in the Materials and methods section. This approach was supported by the overlap described above, as well as by evidence that the amygdala plays a role in addictive behaviours.<sup>35</sup> The results were significant only for the right amygdala ROI in the group of PWS participants but not in controls. Further analyses revealed a significant difference between the participants with PWS and the TD controls in the right amygdala. Specifically, there was greater activation for the caramel odour compared with the tulip odour in the participants with PWS than in the controls [ $p_{\text{corr(FWE)}} < 0.05$ , size = 27,  $t = 2.29$ ,  $(x, y, z) = (15, -1, -10)$ ]. We then extracted the peak activation values for each individual and compared the values for the caramel and tulip odours. Using a linear mixed effects model, we tested the within-subject factor Stimulation type and the between-subject Group factor, Subject being the random effect [R lme4 and mutcomp libraries, the glht (general linear hypotheses test) *post hoc* test]. We found that the participants with PWS had significantly greater activity in the amygdala region than the

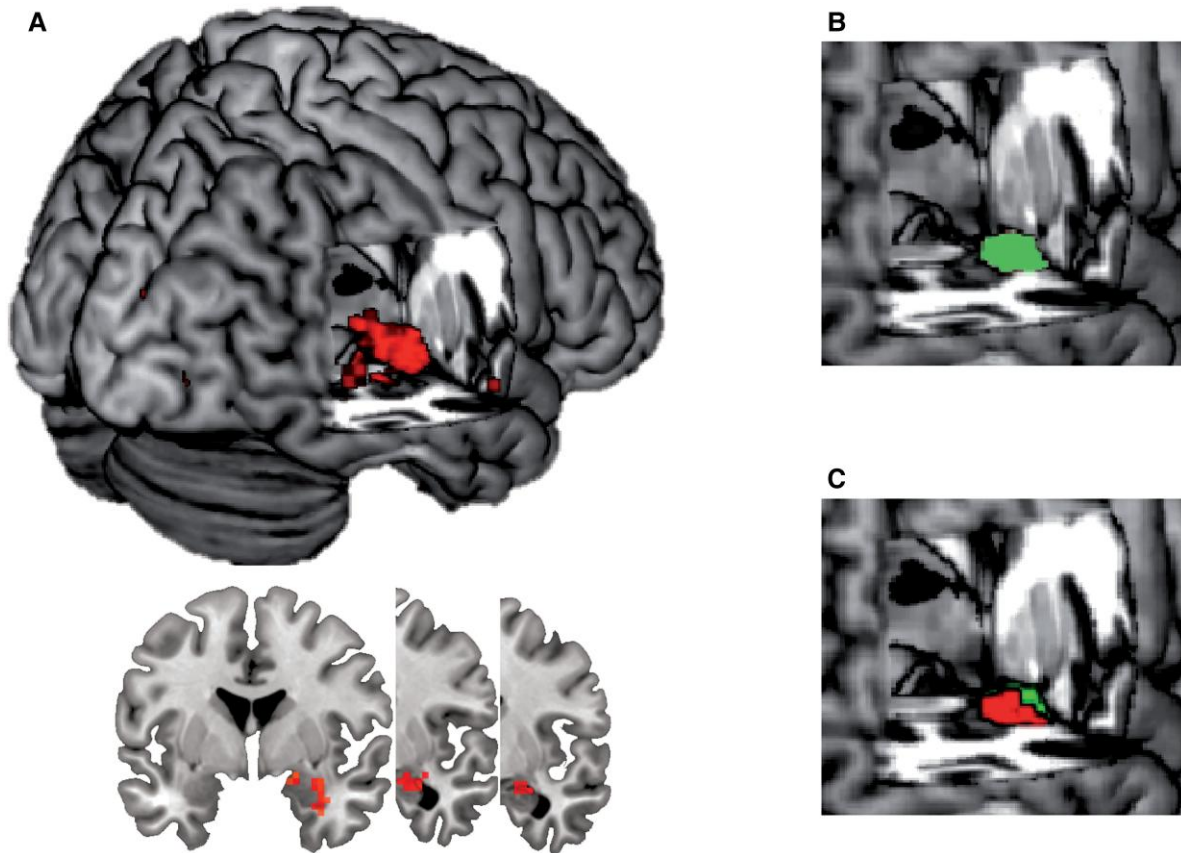
controls when presented with the caramel odour ( $P = 0.02$ ), but there was no significant group difference when they were presented with the tulip odour ( $P = 0.59$ ). In addition, there was a significant difference between the activity for the caramel and tulip odours in the PWS group ( $P = 0.00056$ ), but not in the TD controls ( $P = 0.85$ ; Fig. 3A).

Next, for illustration purposes, we subtracted the values for the tulip odour from those for the caramel odour, and visually compared the results for the two groups. The visual inspection revealed that the mean ‘caramel–tulip’ activity difference was more than five times higher in the PWS group than in the controls (Fig. 3D). At an individual level, the ‘caramel–tulip’ difference was found to be quite variable among the control participants, whereas a clear positive difference could be seen for the majority of the PWS participants, with only one subject manifesting a negative difference (Fig. 3D). By arbitrarily defining difference values between  $-0.5$  and  $0.5$  as statistical noise (about 10% of the global spread), 8 of the 11 controls were seen to have no activity difference, whereas 10 of the 14 PWS subjects had greater activity for the caramel odour and only one had greater activity for the tulip odour. Interestingly, this latter patient also had the lowest HQ (total score 11) and HQ-CT (score 0) scores.

Using a linear mixed effects model, we also tested the fixed within-subject factor Stimulation type and the fixed between-subject Type factor (DEL, UPD and TD) with Subject being the random effect to determine whether there were differences for participants with the DEL or UPD genetic subtype of PWS. It was found that both subtype groups displayed a significant difference between the right amygdala activity peak for the caramel and tulip odours ( $P = 0.013$  for DEL,  $P = 0.006$  for UPD, Fig. 4A). The ‘caramel–tulip’ difference was not significantly different between the subtype groups ( $P > 0.05$ ; Fig. 4B), although the mean value was higher in the DEL group. No correlation between amygdala activity with HQ scores and BMI was found.

## Neuroimaging analysis for PWS clusters

We examined whether the individual differences in activity levels for the two different odours related to clinical scores of hyperphagia in the participants with PWS, specifically the total score and subscores of the HQ, and the total score of the HQ-CT. No significant correlations were found, probably because of the marked heterogeneity in the individual profiles. In order to further examine whether the clinical profiles related to the patterns of brain activity, we used k-means clustering to partition the subjects with PWS into groups according to their clinical scores. The machine learning algorithm detected three clusters with 3–5 subjects and one cluster with a single subject. The three main clusters were separated according to the first dimension (Fig. 5A), which accounted for 84% of the variability in scores; the second dimension only accounted for 11% of the variability. There were strong correlations between the first dimension



**Figure 2 Specific blood oxygen level dependent activity in response to the caramel odour compared with the tulip odour. (A)** Difference between the caramel and tulip odour blocks in participants with PWS in the whole-brain analysis [paired  $t$ -test,  $p_{\text{corr(FWE)}} < 0.05$ , size = 75,  $t = 5.99$ ,  $(x, y, z) = (21, -1, -13)$ ]. The thresholded  $t$ -values are plotted to show activity. This pattern was significantly different compared with the control participants. **(B)** Anatomical location of the right amygdala according to the cytoarchitectonic atlas. **(C)** Overlap between the anatomical position of the right amygdala and the whole-brain activity difference.

and both the total HQ score ( $r = 0.995$ ) and the HQ-CT score ( $r = 0.986$ ).

An ANOVA was run using the groups obtained in the cluster analysis and the ‘caramel–tulip’ amygdala activation difference. The results revealed a significant group effect [ $F(3,10) = 5.59$ ,  $P = 0.016$ ]. *Post hoc* comparisons identified a significant difference between the first and the second clusters ( $P = 0.011$ ; Fig. 5B). The first cluster was found to have a smaller activation difference for the two odours, and it corresponded to the group with the lowest HQ-CT scores (mean: 3.6) and HQ total scores (mean: 17.4); all of the patients in this group had an HQ-CT score lower than 22, which corresponds to the threshold for clinical severity used in clinical trials. In contrast, patients in the second cluster, who had the largest activation differences for the two odours, all had HQ-CT total scores above 22. We also discarded the outlier and conducted the analysis on the three clusters. In this case, we found  $F(2,10) = 7.99$ ,  $P = 0.008$  for the group effect. In the *post hoc* test, the difference between Clusters 1 and 2 was at  $P = 0.006$  and between Clusters 2 and 3 at  $P = 0.05$ .

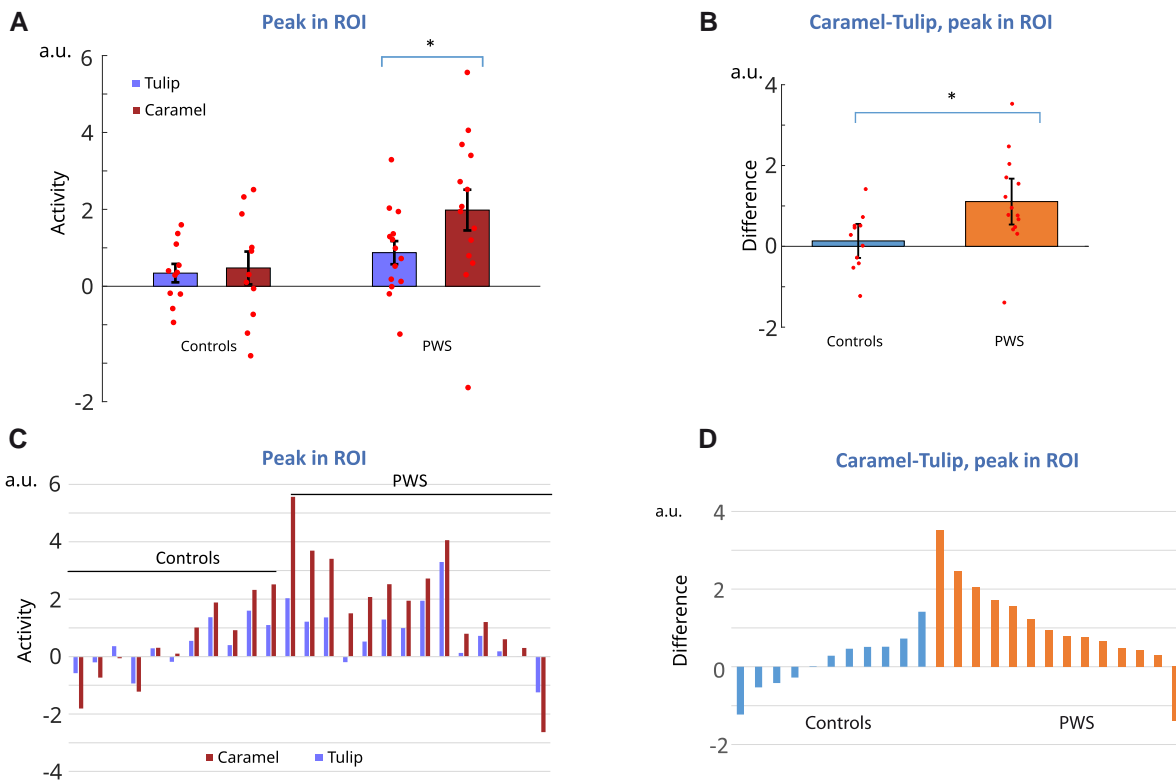
Altogether, our results indicate that there is abnormal, elevated activity in the right amygdala in response to a

food-related odour in patients with PWS. This elevated activation relates to hyperphagia and its severity.

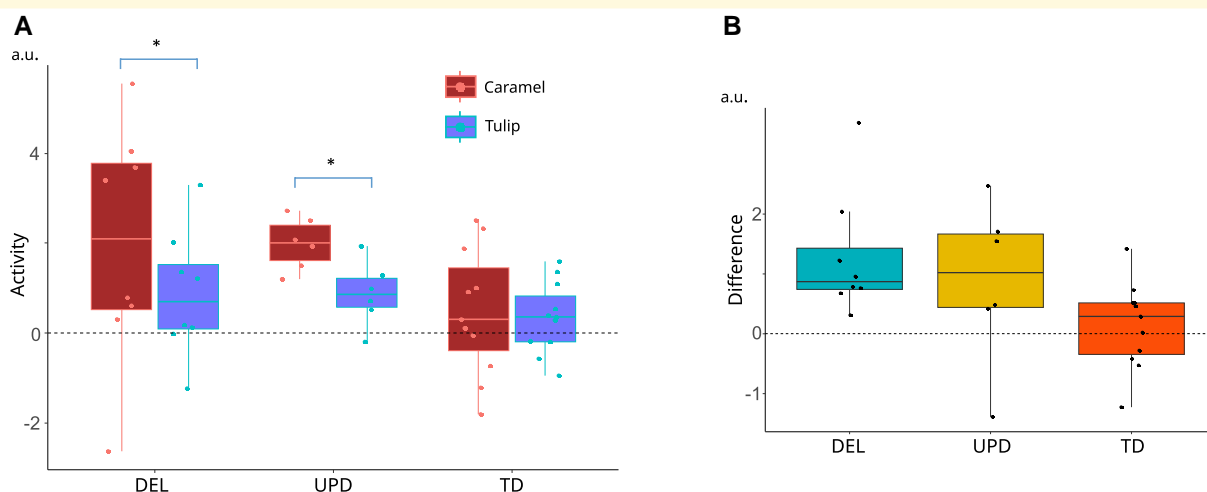
## Discussion

This study is the first to investigate odour-related brain activations in adult patients with PWS using fMRI. The results revealed normal performance on an odour recognition test in patients with PWS, but differences in odour-related brain activations. Specifically, we found that for patients with PWS, a food-related odour (caramel) elicited higher levels of brain activation in the right amygdala compared with an odour unrelated to food (tulip); this odour-related difference was significantly higher in the patients than in age-matched controls. The difference between the patient and control groups was large, with the right amygdala activation being five times higher in the PWS group. Three main clusters of patients were identified with respect to the clinical scores of hyperphagia, which correlated highly with the hyperphagia HQ score ( $r = 0.995$ ) and HQ-CT score ( $r = 0.986$ ). ANOVA demonstrated the

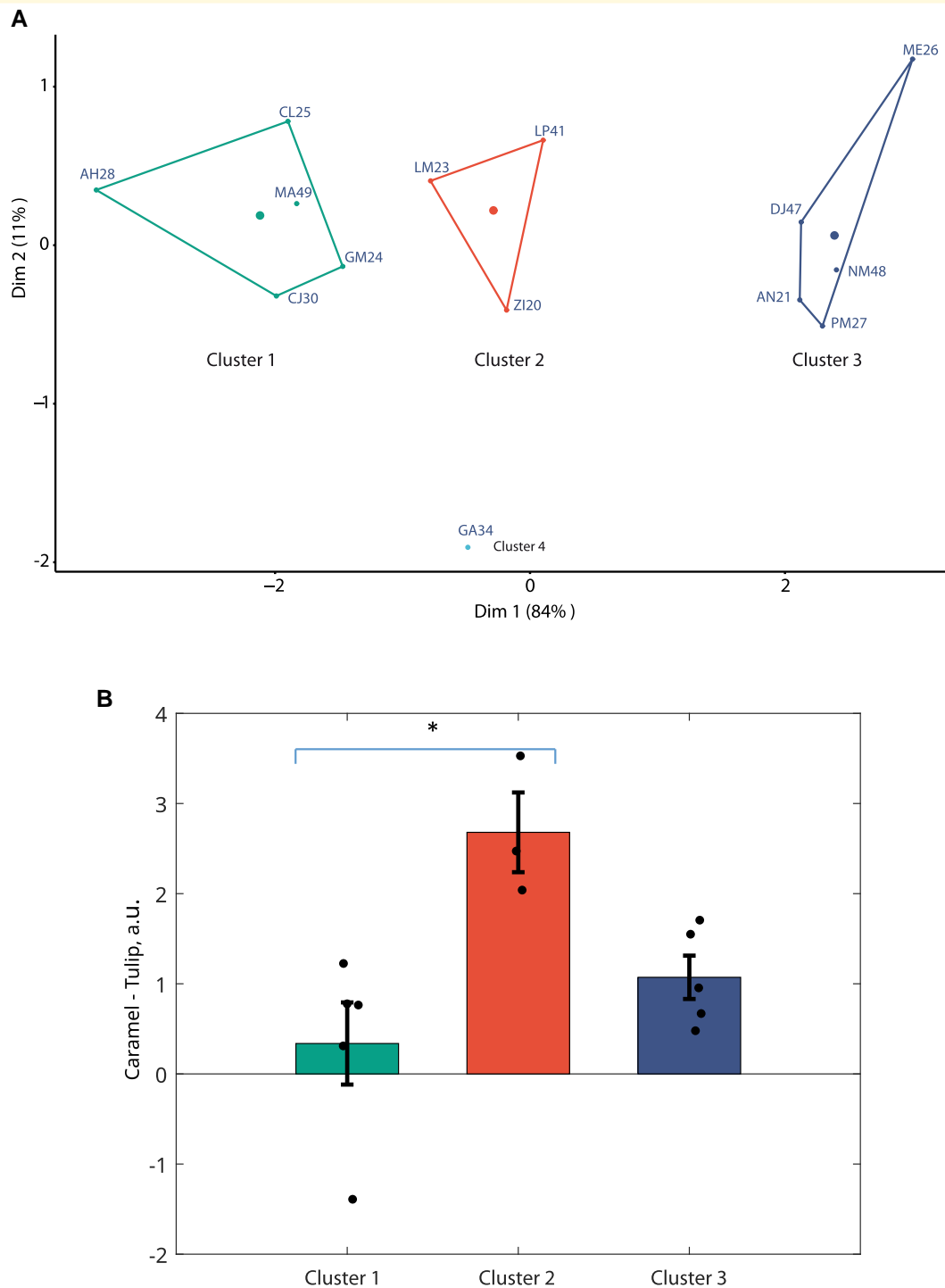




**Figure 3** Activity in the right amygdala for the caramel and tulip odours. **(A)** Peak activity in the right amygdala for the caramel and tulip odours in the PWS and control groups. \* indicates  $P < 0.05$  (glt *post hoc* test,  $P = 0.02$ ). All the activity values in the figures are in arbitrary units provided by SPM. **(B)** Difference between the peak activity in the right amygdala for the caramel and tulip odours ('caramel-tulip'), shown for the PWS and control groups. **(C)** Individual levels of activity for caramel and tulip odours at the peak in the right amygdala for PWS and controls. **(D)** Individual differences for the 'caramel-tulip' right amygdala peak, shown for the participants with PWS and the controls.



**Figure 4** Right amygdala peak activity for participants with different genetic subtypes of PWS and TD controls. **(A)** Right amygdala peak activity for the caramel and tulip odours in participants with the DEL or UPD subtypes of PWS and TD controls. No significant differences were found between the PWS subtype groups. \* indicates  $P < 0.05$  (glt *post hoc* test). **(B)** Difference in amygdala activity in response to the caramel and tulip odours ('caramel-tulip'). There was no significant difference between the PWS subtype groups, although participants with the DEL subtype had the highest odour-related activity difference.



**Figure 5 K-means clustering of scores and activity differences in the right amygdala. (A)** Unsupervised machine learning using k-means clustering detected four clusters of subjects according to their HQ and HQ-CT total scores. There were three main clusters with 3–5 subjects and a fourth cluster with one subject. The three main clusters were divided according to the first dimension, which accounted for 84% of the variability in scores. **(B)** The ‘caramel–tulip’ difference in right amygdala activity for the different clusters of subjects. There was a significant difference between the first and second clusters, as indicated by an asterisk (*post hoc* test,  $P = 0.011$ ). Activity values in the figures are in arbitrary units provided by SPM.

significant effect of clusters on the right amygdala activation, which indicated that the modified activity was related to the degree of hyperphagia.

Our results suggest that there may be a specific alteration of the brain circuits involved in processing food odours in PWS associated with hyperphagia. It is possible

that similar differences may not be apparent for visual processing, which would be in line with our finding that there were no RT differences for food and non-food images in patients with PWS, as also found in our previous study.<sup>38</sup>

Previous articles have described the concept of ‘food addiction’, whereby certain foods with added fats or sugars may activate the reward system in a manner similar to drugs of abuse.<sup>40–42</sup> This may trigger an addiction to food in susceptible individuals. In line with this, it has been found that high-fat, high-sugar foods are frequently consumed during episodes of binge eating,<sup>10,43,44</sup> and there is evidence that they may lead to poor control of eating behaviours.<sup>43,45,46</sup> In addition, foods with added fats and refined carbohydrates are more likely to be intensely craved than fruit and vegetables.<sup>47–49</sup> They are also consumed in greater quantities when people experience negative emotional states.<sup>50,51</sup> Bingeing on these foods leads to changes in the reward system that are seen in other addictive disorders, such as the downregulation of dopamine receptors.<sup>52,53</sup> In our study, caramel was used as the food-related odour, because patients with PWS have a strong preference for sweet foods.<sup>54</sup> As this is a high-sugar food, these addiction-related considerations are of particular relevance.

Previous work has shown that odours are potential cues for addictive behaviours.<sup>47,49,55</sup> Interestingly, the brain circuits involved in processing food odours share many common areas with those activated by cues for addictive substances.<sup>56</sup> The key brain areas implicated in food and related emotional/addictive behaviour are the basal ganglia, the extended amygdala (central medial amygdala, subnucleus of the amygdala, the nucleus accumbens shell and the bed nucleus of the stria terminalis), and the prefrontal cortex. In our study, there was hyperactivation in one of these areas, the right amygdala, in response to a sweet food odour in patients with PWS. This finding may relate to the functional connectivity of the amygdala, which may play a role in detecting the emotional salience of different stimuli, particularly those related to food and stress. In line with this, neuroimaging studies in humans have demonstrated increased amygdala activation in response to high-calorie food cues compared with low-calorie food cues,<sup>57</sup> and also altered functional connectivity between the amygdala and different cortical areas<sup>58</sup> in obese compared with lean subjects at rest. Furthermore, amygdala activity has been associated with scores on the Yale Food Addiction Scale,<sup>35,36</sup> and both the amygdala and the orbitofrontal cortex have been shown to be more highly activated in obese than lean individuals when anticipating the receipt of food.<sup>35</sup> There is evidence that both patients with obesity and those with addictions exhibit augmented activity in the amygdala and ventral striatum in response to reward,<sup>59</sup> thus supporting an overlap between food-related and salience-related circuits.

We found lateralization of the abnormal hyperactivation of the amygdala complex, which was more pronounced in the right hemisphere. An asymmetrical organization of the

amygdala has been reported from structural, molecular and functional aspects.<sup>60</sup> It has been hypothesized that the right amygdala is involved in the rapid detection of emotional stimuli, whereas the left amygdala may be more involved in a more sustained evaluation of stimuli.<sup>61–63</sup> In support of this, Gläscher and Adolphs<sup>64</sup> found enhanced activation of the right amygdala after the subliminal presentation of emotional facial expressions, while the left amygdala showed increased activation when the stimuli were presented supraliminally. In addition, a previous meta-analysis found that right amygdala activity was positively associated with craving.<sup>65</sup> The right-sided activation is in line with the odours, having been presented for a short time and inducing a craving response. Our study demonstrated increased activation of the right amygdala in response to a food-related odour in patients with PWS, similar to the increased activation seen in subjects with obesity or substance addiction.<sup>59</sup> Individuals with PWS also showed greater activation in the right amygdala, compared to the controls, to high-calorie foods compared to non-foods.<sup>66</sup> It is possible that the activations were higher than they would have been for a different food odour with lower sugar content. Lastly, the observation of a greater evoked activity in the right amygdala complex is in agreement with some alterations observed in ASD patients, which shares many similarities with PWS in terms of abnormal processing of emotional stimuli. Indeed, ASD patients present an increased volume in the right amygdala compared to controls<sup>67</sup> as well as its overactivation.<sup>68</sup> Altogether, despite some discrepancies concerning the amygdala functional asymmetry and the variability of its impairment in neuro-psychopathologies, our results support the lateralization of the amygdala alterations in the PWS.<sup>66</sup> However, further studies are needed to establish a strong hypothesis about the functional significance of the hyperactivation of the right hemisphere complex in opposition to the left side.

Patients with PWS have been found to have high levels of ghrelin and OXT, both of which are involved in addictive behaviours. Interestingly, these hormones have specific regulatory effects on the olfactory bulb in addition to their effects on the hypothalamus and limbic system, including the Ventral Tegmental area. For instance, OXT has been found to modulate the activity of mitral cells, projection neurons that directly convey sensory information from the olfactory bulb to the olfactory cortices and the medium amygdala.<sup>69</sup> In addition, there is increasing evidence that OXT modulates sensory processing, with distinct functions in early and higher olfactory brain regions. The OXT abnormalities in PWS may potentially underlie the increased amygdala activations seen in this study through effects on the olfactory processing pathways. In addition, ghrelin abnormalities may also play a role, as there are ghrelin receptors in the olfactory bulb with neural projections (mitral cells) to the amygdala and hypothalamus. There is evidence that this pathway may be involved in regulating feeding behaviour in response to odours,<sup>70</sup> and it may be upregulated in PWS.

The results of the study suggest that interventions to familiarize food odours in patients with PWS may be implemented to decrease hyperactivation of amygdala and therefore improve compulsive feeding as it has been proposed in children with ASD to modulate olfactory emotions.<sup>30</sup>

It is possible to highlight several limitations to our brain imaging study, including the small number of participants. However, given the low incidence of this rare disease (about 1/20 000), this still represents a satisfactory recruitment record. The patients were engaged in a passive task, and even more exciting results would have been obtained if the patients had produced an olfactory discrimination task. However, we must consider the difficulty of engaging these patients who suffer from compulsive behaviours in a functional imaging session, which is stressful for them. Another limitation is the absence of comparison with other pathologies, such as the compulsive behaviour of obese people. Such comparisons will constitute a second step in order to define if these alterations are specific to PWS. However, we found no correlation between BMI values and neural markers. Besides, it seems that ghrelin and hyperphagia are more important hallmarks of PWS than obesity.<sup>2</sup> Indeed, even without increased BMI, patients with PWS display hyperphagia. Thus, despite these limitations in the experimental protocol, we can be largely satisfied and convinced of the results.

## Supplementary material

Supplementary material is available at *Brain Communications* online.

## Ethical statement

The study was approved by the local ethics committee ('Comité de Protection des Personnes Sud-Ouest et Outremer 1', Toulouse Hospital CHU 13687203; National EudraCT 201300437-33). Written informed consent was obtained from each patient's legal guardian or next of kin prior to the study.

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## Competing interests

The authors report no conflicts of interest.

## Data availability

The data that support the findings of this study are available on request from the study Sponsor (Toulouse University Hospital) through the corresponding author. The data are not publicly available due to the quality of personal health data containing information that could compromise the privacy of research participants. Given the nature of the data and in order to comply with the Sponsor's data protection policy, a data-sharing agreement must be established with the Toulouse University Hospital before the access.

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